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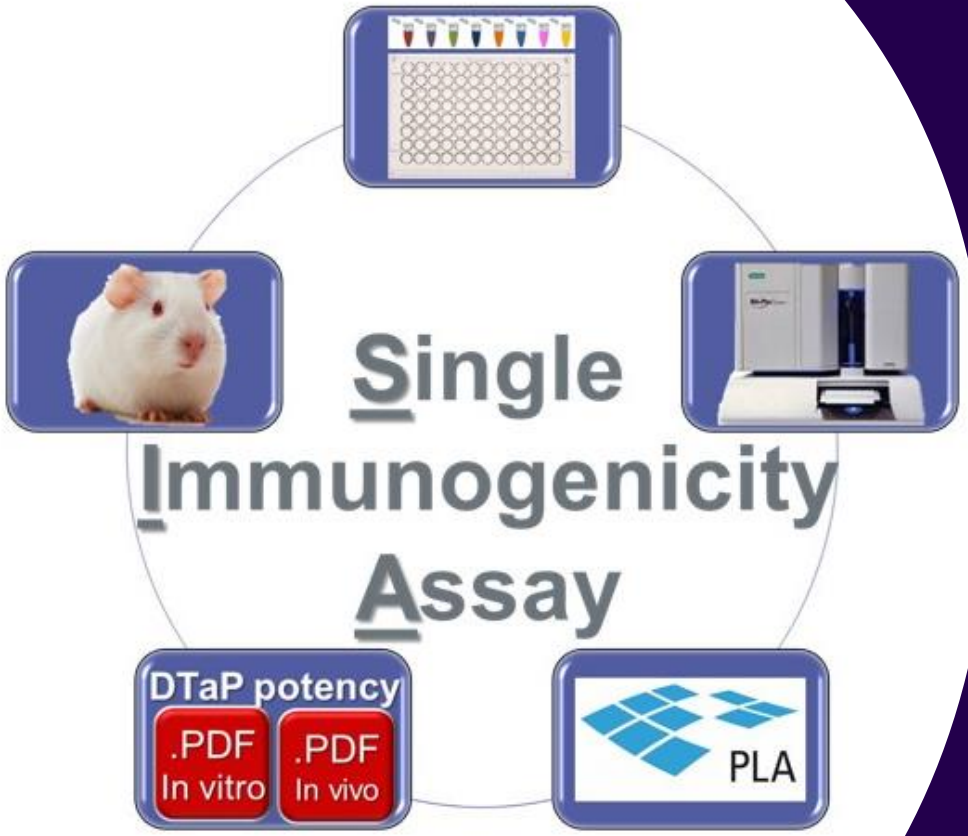

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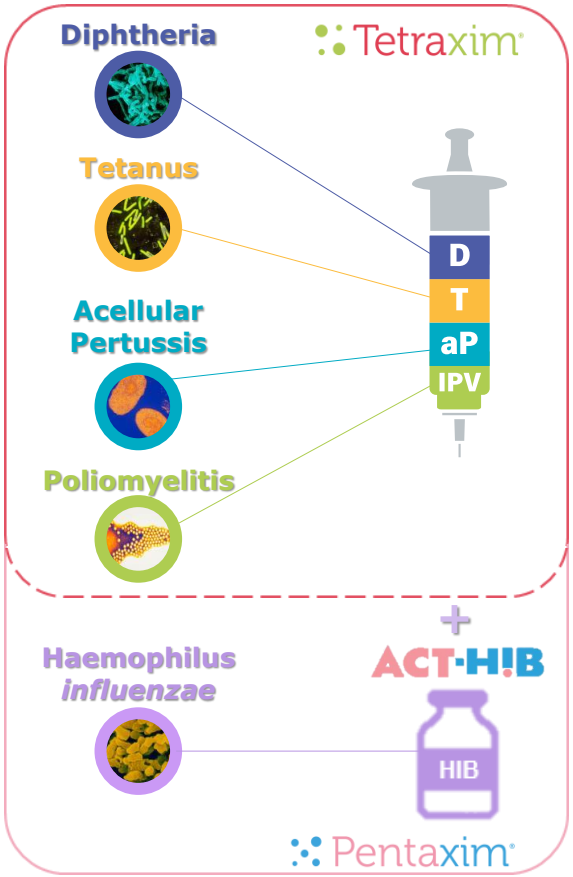
DTaP Single Immunogenicity Assay (SIA)

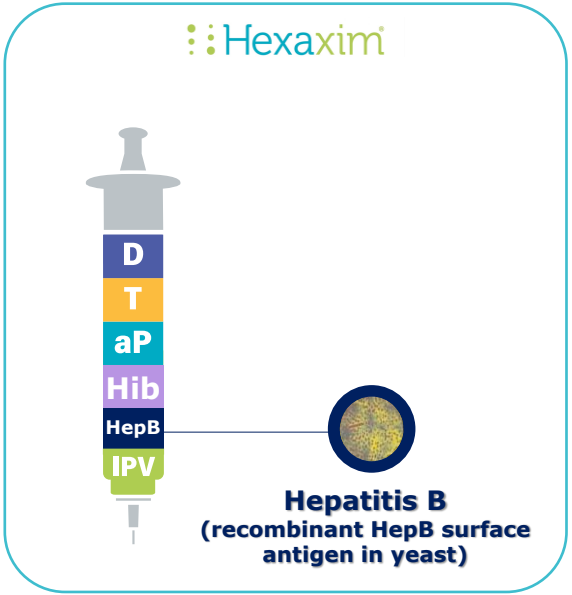
A single-dilution DTaP immunogenicity assay for *AcXim* vaccines routine release and stability testing

HSI – AFSA – 30/01/2025

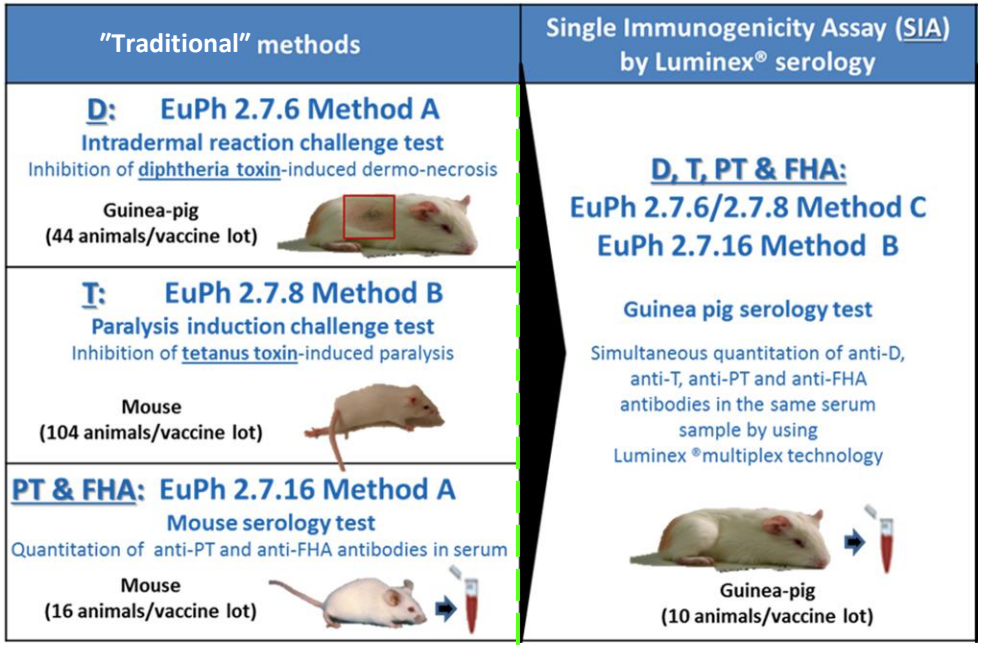
Silvio BANDIERA PhD
AcXim Analytical Product Leader

The products





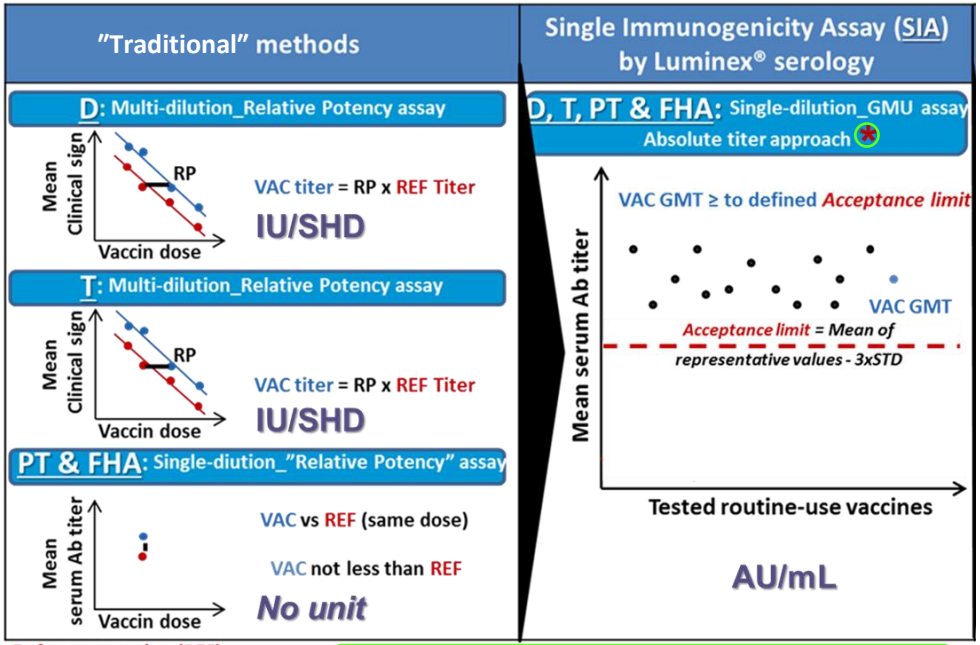
The DTaP potency testing regulatory frame (EU)



Ph. Eur. 2.7.16 Assay of Pertussis Vaccine (acellular): "[…] *For combinations containing pertussis components together with Diphtheria and Tetanus components, the serological assay in guinea pigs can be performed with the same group of animals* […]"

European Directive 2010-63: one of the most stringent ethical and welfare standard for the protection of animals for laboratory use

The change in product acceptance limits



Reference vaccine (REF)
Test vaccine (VAC)
Relative Potency (RP)

* The suitability study is built with a multi-dilution design to choose the single dilution for the final design of the routine test

IU/SHD = International Units by Single Human Dose
GMU = Geometric Mean Unitage
GMT = geometric Mean Titer

The applied SIA implementation strategy_1/2

1) Discuss with HAs and obtain endorsement for applying the serological approach described in Ph.Eur. 2.7.16 for acellular Pertussis to Diphtheria and Tetanus guinea pig serology

I. […] *Once the suitable dilutions have been confirmed for a given vaccine, it is recommended, in accordance with 3R principles (Replacement, Reduction, Refinement), to apply a simplified model such as a* ***single dilution*** […]

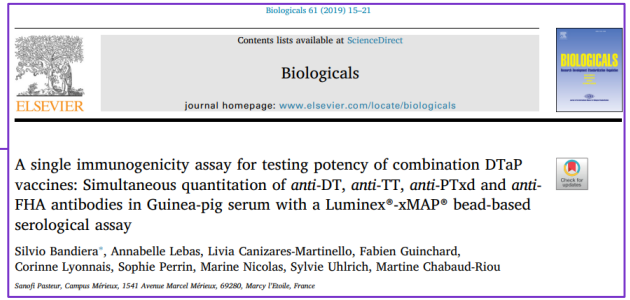
II. […] ***The assay results can be expressed****:*

– *either as a ratio of the geometric mean titer (GMT* […] *relative potency assay)* […]

– *or* ***directly as a GMT of antibodies induced by the test vaccine*** *(geometric mean unit assay or GMU assay* […]

2) Perform the ICHQ2R1-based validation of *in vitro* anti-DTaP antibodies (Ab) simultaneous quantitation by multiplex Luminex™ technology:

- Specificity
- Linearity
- Accuracy
- Precision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A single immunogenicity assay for testing potency of combination DTaP vaccines: Simultaneous quantitation of *anti*-DT, *anti*-TT, *anti*-PTxd and *anti*-FHA antibodies in Guinea-pig serum with a Luminex®-xMAP® bead-based serological assay

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The applied SIA implementation strategy_2/2

3) Perform a comprehensive SIA *in vivo* suitability study (according to Ph.Eur. indications)

I. Calibrate a SIA internal reference standard vaccine *vs* D and T international standards (IU/SHD)

II. Perform multiple dilutions testing in double, traditional methods and SIA, on at least 15 commercial batches, possibly including clinical batches, in order to:

a) Verify linear and parallel dose-response profile on at least 3 consecutive doses

b) Verify SIA IU/SHD titers meet traditional methods' acceptance limits, i.e. SIA at least as discriminatory as traditional D and T challenge methods

c) Choose a dilution in the linear and parallel SIA dose-response range for the single dilution approach

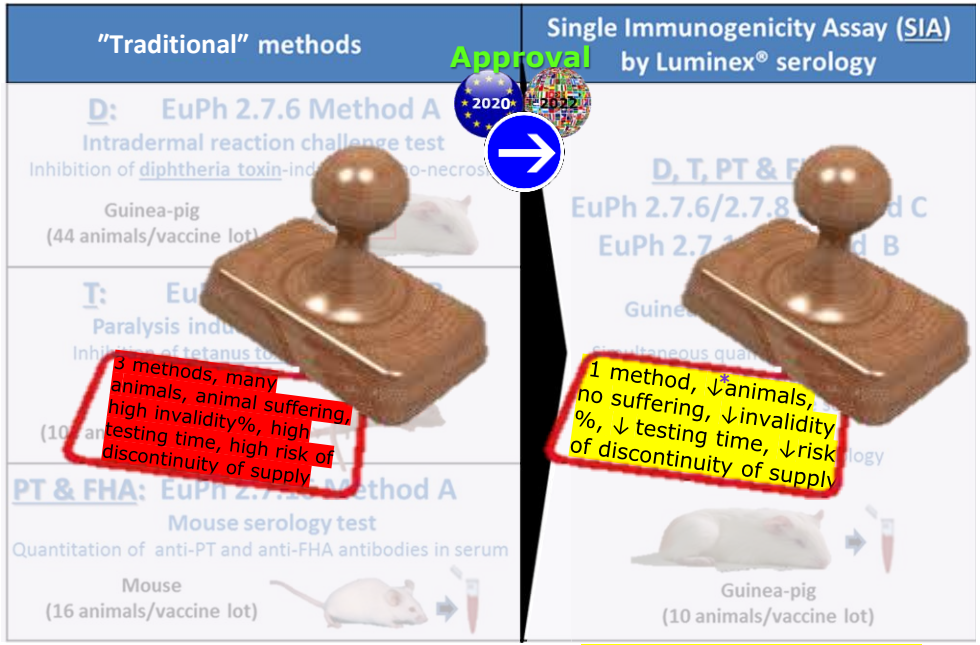
III. Establish consistency-based preliminary acceptance limits on the Ab titers measured at the chosen single dilution (i.e. lowest dilution, i.e. highest concentration, in the validated range for all antigens)

4) Plan and execute the SIA Life Cycle Management

I. Re-evaluate appropriateness of established preliminary acceptance limits once more release data (ex. ≥ 100 batches) have been obtained

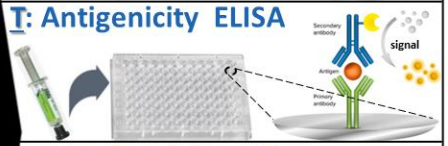
II. Periodically conduct SIA multiple dilution testing to confirm validated suitable dose-range (plus use SIA multiple dilution testing in case of major manufacturing process changes)

SIA measured advantages to date



*** ↓105k less animals estimated over 3 years for Hexaxim only**

SIA is the present: what's next?

PAST (traditional methods)	PRESENT	FUTURE
Previous methods	**Single Immunogenicity Assay (SIA) by Luminex® serology**	**Antigenicity by Sandwich ELISAs**
D: EuPh 2.7.6 Method A Intradermal reaction challenge test Inhibition of diphtheria toxin-induced dermo-necrosis Guinea-pig (44 animals/vaccine lot) 	**D, T, PT & FHA:** **EuPh 2.7.6/2.7.8 Method C** **EuPh 2.7.16 Method B** Guinea pig serology test Simultaneous quantitation of anti-D, anti-T, anti-PT and anti-FHA antibodies in the same serum sample by using Luminex ®multiplex technology  Guinea-pig (10 animals/vaccine lot)	**D: Antigenicity ELISA** 
T: EuPh 2.7.8 Method B Paralysis induction challenge test Inhibition of tetanus toxin-induced paralysis Mouse (104 animals/vaccine lot) 		**T: Antigenicity ELISA** 
PT & FHA: EuPh 2.7.16 Method A Mouse serology test Quantitation of anti-PT and anti-FHA antibodies in serum Mouse (16 animals/vaccine lot) 		**PT: Antigenicity ELISA**  **FHA: Antigenicity ELISA** 

Acknowledgements

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Thank you

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